Intact Protein LC-FTICR MS Identifies Dynamics of Post Translational Modifications to Calmodulin in Macrophages

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Methods

FTICR MS and capillary RPLC-FTICR MS were performed as described. Details are in the Supporting Information. Calmodulin (CaM) was modified with peroxynitrite in the presence of a cell lysate. The data were acquired using an APEX-Q FTICR mass spectrometer that incorporates an ion funnel interface, various ion transfer, selection and storage multipoles.

Results

Figure 1. Example workbench scouting sample prepared for LC-FTICR intact protein analysis.

Figure 2. A substantial loss of nitrotyrosine post incubation with the lysate was observed; this is in agreement with earlier immunoblot data (i.e., Figure 4). The exposure to macrophage lysate also induces a substantial reduction in the biological implication for this is that when LPS causes a bacteriocidal burst of radicals within the macrophage, nitrotyrosine becomes modified CaM. The specificity of this cleavage may function to regulate oxidatively modified CaM. Des(Lys)calmodulin, lacking the carboxy terminal lysine previously detected after incubation of calmodulin with cytosolic extracts of brain and anterior pituitary region [4].

Conclusions

To further clarify these results and quantify nitration, denitration and adduct formation, we find a concomitant C-terminal lysine cleavage occurring selectively to modified CaM. The specificity of this cleavage may function to regulate oxidatively modified CaM. Des(Lys)calmodulin, lacking the carboxy terminal lysine previously detected after incubation of calmodulin with cytosolic extracts of brain and anterior pituitary region [4].

Acknowledgements

References

5. Purify...